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Pain in the Ehlers–Danlos syndromes: Mechanisms, models, and challenges

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Abstract

Chronic pain is one of the most common, yet poorly studied, complaints in people suffering from Ehlers–Danlos syndromes (EDS). This heterogeneous group of heritable connective tissue disorders is typically characterized by skin hyperextensibility, joint hypermobility, and generalized connective tissue fragility. Most EDS types are caused by genetic defects that affect connective tissue biosynthesis, thereby compromising collagen biosynthesis or fibrillogenesis and resulting in a disorganized extracellular matrix. Even though chronic pain is a major source of disability, functional impairment, and psychosocial suffering in EDS, currently used analgesics and other treatment strategies provide inadequate pain relief and thus represents an important unmet medical need. An important contributor to this is the lack of knowledge about the underlying mechanisms. In this narrative review, we summarize the current understanding of pain and the associated mechanisms in EDS based on clinical studies focusing on questionnaires and experimental pain testing as well as studies in animal models of EDS. In addition, we highlight the challenges, gaps, and opportunities in EDS-pain research.

KEYWORDS
Ehlers–Danlos syndromes, extracellular matrix, hypermobility spectrum disorders, pain

1 | THE EHLERS–DANLOS SYNDROMES AND HYPERMOBILITY SPECTRUM DISORDERS, MULTISYSTEMIC DISORDERS

The Ehlers–Danlos syndromes (EDS) comprise a group of rare heritable connective tissue disorders, clinically hallmarked by generalized joint hypermobility, skin hyperextensibility and fragility, abnormal wound healing, easy bruising, and widespread connective tissue friability (Malfait et al., 2020). Originally suspected to be a disorder affecting the collagen "wickerwork," based on electron microscopy studies on skin biopsies from affected individuals (Jansen, 1955), early biochemical and genetic studies indeed identified defects in the primary structure of fibrillar collagens (collagens type I, III and V) or their modifying enzymes (lysyl hydroxylase 1, procollagen I amino-proteinase) in individuals with different variants of EDS (Beighton et al., 1988; Beighton, De Paepe, Steinmann, Tsipouras, & Wenstrup, 1998). These discoveries laid the foundation for the 1998 Villefranche EDS Nosology, which was used for over two decades and included six EDS types (classical, hypermobile, vascular, arthrochalasis, kyphoscoliotic, and dermatosparaxis EDS; Beighton et al., 1998). With advances in molecular techniques, defects affecting other extracellular matrix (ECM) molecules (e.g., the glycoprotein tenascin X, enzymes involved in glycosaminoglycan biosynthesis of proteoglycans, the collagen-chaperone FKBP22, etc.) were identified, which vastly expanded the molecular and clinical heterogeneity of these syndromes, necessitating a revision of the EDS classification (Malfait et al., 2017). To date, 13 distinct EDS types are recognized, 12 of which have been molecularly elucidated, caused by defects in 20 different genes (Malfait et al., 2020; Malfait et al., 2017; Table 1). All these EDS-related defects compromise collagen biosynthesis,

TABLE 1 Overview of the different types of EDS, their transmission pattern, underlying genetic defects, and main clinical manifestations

Pathogenic mechanism	EDS type	IP	Gene	Protein	Major clinical criteria
Defects in collagen primary structure and collagen processing	Classical EDS (cEDS)	AD	*COL5A1* *COL5A2*	α1(V) procollagen chain α2(V) procollagen chain	Skin hyperextensibility with atrophic scarring Generalized joint hypermobility
	Classical EDS (cEDS)	AD	*COL1A1*	α1(I) procollagen chain p.(Arg312Cys)	Skin hyperextensibility with atrophic scarring Generalized joint hypermobility Arterial rupture at young age
	Vascular EDS (vEDS)	AD	*COL3A1*	α1(III) procollagen chain	Arterial rupture at young age Spontaneous sigmoid colon perforation in the absence of known colon disease Uterine rupture during third trimester of pregnancy Carotid-cavernous sinus fistula (in the absence of trauma)
	Arthrochalasia EDS (aEDS)	AD	*COL1A1* *COL1A2*	α1(I) procollagen chain α2(I) procollagen chain	Congenital bilateral hip dislocation Severe generalized joint hypermobility with multiple dislocations Skin hyperextensibility
	Dermatosparaxis EDS (dEDS)	AR	*ADAMTS2*	procollagen I amino-proteinase (ADAMTS2)	Extreme skin fragility with congenital or postnatal tears Craniofacial features Progressively redundant, lax skin with excessive skin folds Increased palmar wrinkling Severe bruisability Umbilical hernia Postnatal growth retardation with short limbs Perinatal complications related to tissue fragility
	Cardiac-valvular EDS (cvEDS)	AR	*COL1A2*	α2(I) procollagen chain (total absence)	Severe progressive cardiac-valvular insufficiency Skin involvement Joint hypermobility
Defects in collagen folding and collagen cross-linking	Kyphoscoliotic EDS (kEDS-*PLOD1*)	AR	*PLOD1*	Lysyl hydroxylase 1	Congenital muscle hypotonia Congenital or early onset kyphoscoliosis Generalized joint hypermobility with (sub) luxations
	Kyphoscoliotic EDS (kEDS-*FKBP14*)	AR	*FKBP14*	FK506-binding protein 22 kDa (FKBP22)	
Defects in structure and function of myomatrix, the interface between muscle and ECM	Classical-like EDS type 1 (clEDS1)	AR	*TNXB*	Tenascin X	Skin hyperextensibility with velvety skin texture and absence of atrophic scarring Generalized joint hypermobility Easy bruisable skin/spontaneous ecchymoses
	Myopathic EDS (mEDS)	AD/AR	*COL12A1*	α1(XII) procollagen chain	Congenital muscle hypotonia and/or muscle atrophy Joint contractures Joint hypermobility

TABLE 1 (Continued)

Pathogenic mechanism	EDS type	IP	Gene	Protein	Major clinical criteria
Defects in glycosaminoglycan biosynthesis	Spondylodysplastic EDS (spEDS-*B4GALT7*)	AR	*B4GALT7*	Galactosyltransferase I (β4GalT7)	Short stature (progressive in childhood) Muscle hypotonia (ranging from severe congenital to mild later-onset) Bowing of limbs Skeletal dysplasia
	Spondylodysplastic EDS (spEDS-*B3GALT6*)	AR	*B3GALT6*	Galactosyltransferase II (β3GalT6)	
	Musculocontractural EDS (mcEDS-*CHST14*)	AR	*CHST14*	Dermatan 4-*O*-sulfotransferase-1 (D4ST1)	Congenital multiple contractures (typically adduction/flexion contractures and talipes equinovarus) Craniofacial features Skin hyperextensibility, easy bruising, skin fragility with atrophic scars Increased palmar wrinkling
	Musculocontractural EDS (mcEDS-*DSE*)	AR	*DSE*	Dermatan sulfate epimerase-1 (DS-epi1)	
Defects in complement pathways	Periodontal EDS (pEDS)	AD	*C1R* *C1S*	C1r C1s	Severe and intractable early-onset periodontitis Lack of attached gingiva Pretibial plaques
Defects in intracellular processes	Spondylodysplastic EDS (spEDS-*SLC39A13*)	AR	*SLC39A13*	Zrt/Irt-like protein 13 (ZIP13)	Short stature (progressive in childhood) Muscle hypotonia (ranging from severe congenital to mild later-onset) Bowing of limbs Skeletal dysplasia
	Brittle cornea syndrome (BCS)	AR	*ZNF469* *PRDM5*	Zinc finger protein 469 (ZNF469) PR domain zinc finger protein 5 (PRDM5)	Thin cornea with/without rupture Early-onset progressive keratoconus and/or keratoglobus Blue sclerae
Unclassified	Classical-like EDS type 2 (clEDS2)	AR	*AEBP1*	Adipocyte enhancer-binding protein (AEBP1)	Skin hyperextensibility with atrophic scarring Generalized joint hypermobility Foot deformities Early-onset osteopenia
Unknown	Hypermobile EDS (hEDS)	?	?	?	Generalized joint hypermobility Systemic manifestations of generalized connective tissue disorder Positive family history Musculoskeletal complaints and pain Exclusion of other EDS types and other joint hypermobility-associated conditions

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; IP, inheritance pattern.

fibrillogenesis, and/or supramolecular organization of collagen fibrils in the ECM at some level, with alterations in the physical properties of the ECM in essentially all tissues and organs. This explains the pleiotropic and multisystemic nature of these conditions. Depending on the EDS type and underlying genetic alteration, individuals with EDS may suffer from a range of signs and symptoms, including, but not limited to, cardiovascular complications (e.g., arterial aneurysms and dissections, cardiac-valvular problems), pulmonary complications (e.g., pneumothorax, lung hypoplasia, restrictive lung disease), ruptures of hollow organs, internal organ prolapses, surgical complications (e.g., incisional hernia), musculoskeletal complications (e.g., spinal and pectus deformities, congenital hip dislocations, joint contractures, muscle hypotonia), ophthalmological problems (e.g., refractory problems, glaucoma, corneal clouding), and urogynecological complications (e.g., pelvic organ prolapses, hydronephrosis, cryptorchidism; Table 1).

Despite major advances in the discovery of genetic alterations underlying many EDS types, the molecular pathways linking these genetic changes to the phenotypes remain largely elusive. In addition, the hypermobile type of EDS (hEDS), presumably the most common type, remains molecularly unexplained to date (Malfait et al., 2017). The Villefranche nosology initially defined hEDS as generalized joint hypermobility with or without skin manifestations, joint pain, dislocations, and/or a positive family history (Beighton et al., 1998), but the criteria were only vaguely defined and the clinical boundaries of hEDS overlapped with those of joint hypermobility syndrome (JHS), a condition associated with joint hypermobility and musculoskeletal and systemic symptoms, as defined by the revised Brighton criteria (Grahame, Bird, & Child, 2000). The lack of a genetic marker for either condition further contributed to the confusion about the terms JHS and hEDS among researchers and clinicians. Eventually, they were found to be so similar that they should be considered the same condition, until genetic studies could determine otherwise (Tinkle et al., 2009). As a result, the terms hEDS and JHS were often used interchangeably, both in routine clinical care and in clinical research studies. To tackle this problem of terminology and the ensuing confusion, the revised 2017 EDS classification now emphasizes that among individuals with otherwise unclassified joint hypermobility, the term hEDS should be limited to individuals with features indicative of a systemic and/or Mendelian connective tissue disorder (Malfait et al., 2020). Individuals with symptomatic joint hypermobility who do not fulfill the 2017 diagnostic criteria for hEDS (and who do not have signs and symptoms of another joint hypermobility-associated condition) are now considered to fall into the group of "Hypermobility Spectrum Disorders" (HSD; Castori et al., 2017), and the term JHS is no longer used. Like hEDS, HSD are a poorly recognized group of connective tissue disorders that involve a spectrum ranging from hypermobility affecting only one or few joints, to generalized joint hypermobility with subluxations, and dislocations. Besides obvious consequences of complications of joint hypermobility, both hEDS and HSD can be associated with a multitude of extra-musculoskeletal functional manifestations, including chronic fatigue, pelvic floor problems, bladder dysfunction, various dysautonomic features, alterations of the immune system (including mast cell activation syndrome), gastro-intestinal dysfunction, behavioral disturbances, and psychological distress (Castori et al., 2017; Malfait et al., 2020; Tinkle et al., 2017). These concurring manifestations are now recognized, with different levels of evidence, as "joint hypermobility-associated comorbidities," but the biological connection to tissue alterations is not clear (Malfait et al., 2020). The prevalence of these comorbidities in other molecularly defined types of EDS has not been documented.

Throughout this narrative review, we will use the terms hEDS and JHS/HSD, but it must be considered that most published studies included still use the old JHS and hEDS criteria and terminology, and often do not make the distinction between hEDS and JHS.

2 | PAIN IN EDS AND HSD: A CHALLENGING PROBLEM

One of the major complaints of individuals suffering from EDS and JHS/HSD is pain (Castori et al., 2013; Chopra et al., 2017; Hakim & Grahame, 2004; Syx, De Wandele, Rombaut, & Malfait, 2017). Defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al., 2020), pain is a short-term adaptive strategy to protect the human body from (potentially) harmful situations and to allow healing of injured tissues. Nociceptive pain (Figure 1a), which is pain directly related to injury or tissue damage, such as a cut or a burn, is usually short-lasting, less than 3–6 months. There are numerous potential causes of acute, nociceptive pain in EDS and JHS/HSD. Depending on the type, these may for instance include subluxations and dislocations of large and small joints, soft tissue injury, surgical interventions, muscle or tendon rupture, skin tears, abnormal wound healing, repetitive bruising, pneumothorax, and internal organ prolapses (Malfait et al., 2020). Pain can also arise from damage to the somatosensory system itself, which is then defined as neuropathic pain, for instance pain caused by nerve entrapments. However, when pain persists for more than 3–6 months or resurfaces after the tissue damage has resolved, neuroplastic changes in the pain processing pathways (Figure 1b) may lead to a hypersensitive state of the somatosensory system, a phenomenon called *central sensitization* (Basbaum, Bautista, Scherrer, & Julius, 2009; Latremoliere & Woolf, 2009; Woolf, 2011). Central sensitization is the outcome of changes in pain-signaling neurons (such as alterations in gene transcription and protein expression in the sensory neurons) and nonneuronal cells (such as satellite glial cells, immune cells, and astrocytes), spinal hyperexcitability, increased activity of pain facilitatory pathways, malfunctioning of descending pain inhibitory pathways resulting in dysfunctional endogenous analgesia, and altered sensory processing in the brain (Basbaum et al., 2009; Latremoliere & Woolf, 2009) and is clinically expressed as allodynia and/or (primary or secondary (generalized)) hyperalgesia (Figure 1c; Latremoliere & Woolf, 2009). When this happens, pain outlives its usefulness as an acute warning system and instead becomes chronic and debilitating. Chronic musculoskeletal pain has long been recognized to be a major complaint in JHS and it was included in the diagnostic criteria (Grahame et al., 2000; Kirk, Ansell, & Bywaters, 1967). For EDS, Sacheti

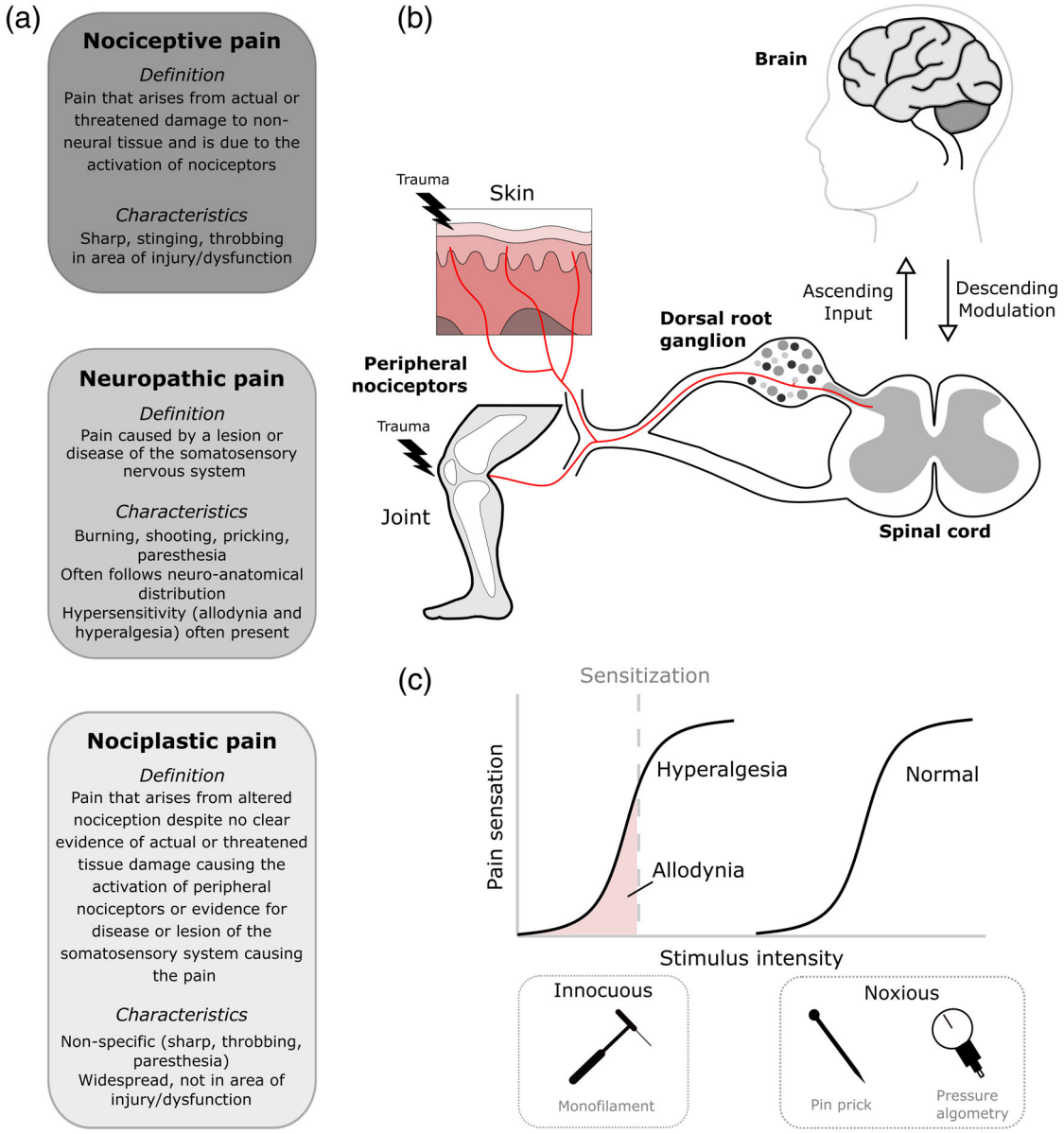

FIGURE 1 Pain mechanisms overview. (a) The three major divisions of pain according to the International Association for the Study of Pain (IASP) with their definition and characteristics. (b) In its most essential form, pain equals nociception, which is caused by the activation of specialized sensory neurons, the nociceptors, with free nerve endings in the skin, joints, muscles, bones, and viscera, by a potentially harmful stimulus of mechanical, thermal, or chemical origin. Two major classes of nociceptors can be recognized: medium diameter myelinated Aδ afferents that mediate acute, well-localized "first" or fast pain and small diameter unmyelinated C fibers that convey poorly localized, "second" or slow pain. The cell bodies of these nociceptors are located within the dorsal root ganglia (DRG). The sensory information from the peripheral free nerve endings is then transferred via the DRG to the spinal cord and further up to higher brain centers where it is analyzed and, if necessary, an appropriate protective response is initiated. The central nervous system (CNS) responds with a change in sensitivity of the nociceptive system by the release of neurotransmitters, by neuroplastic adaptions and by modulating the pain signal within the pain pathways located in the nervous system. This leads to either *facilitation* or *inhibition* of the pain. (c) In normal circumstances, the pain sensation is proportional to the intensity of the noxious stimulus. However, in the case of sensitization an increased sensitivity is observed to normally innocuous stimuli (allodynia) and/or an exaggerated response to noxious stimuli (hyperalgesia)

et al. (1997) were the first to report a 90% incidence of chronic pain in 51 patients with different EDS types. This incidence was later confirmed in a few other questionnaire studies (Benistan & Martinez, 2019; Rombaut, Malfait, Cools, De Paepe, & Calders, 2010; Voermans, Knoop, Bleijenberg, & van Engelen, 2010). These reports echo clinical experience that chronic pain is one of the most frequent complaints of patients affected by EDS, especially hEDS. This is also reflected in the fact that (chronic) pain (described as either "joint pain," "musculoskeletal pain," or "chronic widespread pain" lasting for longer than 3 months), was/is part of the diagnostic criteria for hEDS, both in the Villefranche (Beighton et al., 1998) and the 2017 EDS classification (Malfait et al., 2017).

Relief from pain is what drives people to seek medical care, but available treatments provide modest relief at best, and are associated with undesirable adverse events and health risks (Syx et al., 2017). The chronic, widespread pain in EDS and JHS/HSD is associated with high use of analgesics, surgery, and physical therapy (Castori et al., 2012; Castori et al., 2013; Chopra et al., 2017; Demes, McNair, & Taylor, 2020; Sacheti et al., 1997; Schubart, Schilling, Schaefer, Bascom, & Francomano, 2019; Syx et al., 2017). If left un- or inadequately treated, it often has a detrimental effect on physical, social, and emotional function in affected individuals, with a substantial deterioration of their quality of life (Bovet, Carlson, & Taylor, 2016; Johannessen, Reiten, Lovaas, Maeland, & Juul-Kristensen, 2016; Scheper et al., 2016). Yet, despite being a major problem in daily clinical practice, surprisingly little research has been done on the prevalence, characteristics, etiology, and mechanisms of pain in EDS and JHS/HSD, even though several tools are available for experimental pain testing in humans (summarized in Table 2).

Most of the studies that have investigated pain in EDS or JHS/HSD focused on adult females with hEDS, JHS, or mixed hEDS/JHS diagnoses, likely reflecting the relative ease of studying a substantial patient sample size necessary for statistical analysis within this group. This complicates studies investigating the molecular pathomechanisms underlying pain in EDS or JHS/HSD, since the genetic basis of hEDS/JHS remains unknown. This also entails that very little is known about pain prevalence and mechanisms in pediatric, adolescent, and male EDS and JHS/HSD populations, nor in individuals affected with another type of EDS. No pathophysiological pain model has been identified yet for EDS, although theoretical arguments and some evidence has been presented for several types of mechanisms, including nociceptive pain, neuropathic pain (Raja et al., 2020), and central sensitization with the development of nociplastic pain (Figure 1a). In many cases, however, multiple etiologies of pain may co-exist.

3 | CHARACTERISTICS AND MECHANISMS OF PAIN IN HEDS AND JHS/HSD

People with hEDS or JHS/HSD are predisposed to dislocations and repetitive soft-tissue traumas, such as overstretching and tearing of ligaments and tendons (Rombaut, De Paepe, Malfait, Cools, & Calders, 2010; Rombaut et al., 2012), due to their ligamentous laxity and joint instability, lack of proprioceptive acuity, and reduced muscle strength and endurance. In addition, microtrauma in the frail connective tissues may cause subclinical damage that is often not supported by a recognized history of trauma. Both micro- and macrotrauma in the joints, tendons, and ligaments may further diminish proprioceptive acuity, increasing the likelihood of additional injuries.

Nociceptive pain directly related to these injuries (often triggered by external factors such as sports injuries or surgery; Castori et al., 2013) is frequently encountered in hEDS and JHS. This type of pain is felt as an acute and localized symptom, usually in relation to joint trauma, or as "growing pains," often during the first or second decade of life. Arthralgia is frequently described in weight-bearing joints such as hips, knees, and ankles, in joints involved in repetitive tasks, such as shoulders, wrists and hands, and in the neck, back and temporomandibular joints (Benistan & Martinez, 2019; Rombaut, Malfait et al., 2010; Tinkle & Levy, 2019). Myalgias are also commonly reported at this stage, with tender myotendinous insertion points and muscle tension (Rombaut, Malfait et al., 2010). Muscle cramps, enthesopathies, such as tendinitis, tenosynovitis and fasciitis, and nerve entrapment syndromes can add to the localized musculoskeletal pain (Aktas, Ofluoglu, & Albay, 2008; Granata et al., 2013; Rombaut, Malfait et al., 2010). Other contributors to nociceptive pain in hEDS and JHS/HSD may include reduced bone mass (Banica et al., 2020; Dolan, Arden, Grahame, & Spector, 1998) and premature osteoarthritis (Jonsson et al., 2009), but the impact of the latter two on the pain phenotype remains to be elucidated.

Besides nociceptive pain, a few questionnaire studies provided evidence for a neuropathic component to hEDS- or JHS-related pain (Benistan & Martinez, 2019; Camerota, Celletti, Castori, Grammatico, & Padua, 2011; Rombaut et al., 2015; Voermans, Knoop, & van Engelen, 2011), although this could not be confirmed in another questionnaire study (Di Stefano et al., 2016). One case report and a study in a small series of hEDS/JHS patients ($n = 20$), three vascular EDS, and one classical EDS patient described decreased intraepidermal nerve fiber density, which is a sign of small fiber neuropathy (Cazzato et al., 2016; Pascarella et al., 2016). Nevertheless, comprehensive cutaneous innervation data on other molecularly solved EDS types are currently not available, thereby representing a gap in the study of pain in EDS.

Pain in EDS gradually becomes less localized and takes on a widespread and chronic character. Common additional complaints in this stage include burning sensations, peripheral paresthesias, generalized hyperalgesia, allodynia, and hypersensitivity to various other stimuli, such as light, sound and odors (Benistan & Martinez, 2019; Castori et al., 2013; Syx et al., 2015). In this stage, the pain distribution and description often overlap with fibromyalgia (Tinkle & Levy, 2019). Besides musculoskeletal pain, other pain phenotypes, especially headaches, abdominal pain, pelvic girdle pain, and dysmenorrhea are frequently reported in individuals suffering from hEDS or JHS/HSD (Ali, Andrzejowski, Kanakaris, & Giannoudis, 2020; Chopra et al., 2017; Fikree, Chelimsky, Collins, Kovacic, & Aziz, 2017; Rombaut, Malfait et al., 2010). Also, in common with other chronic pain syndromes, overlapping conditions such as chronic fatigue, sleep disturbances, irritable bowel syndrome, depression and anxiety often co-exist (Bulbena et al., 2017; Fikree et al., 2017; Hakim, De Wandele, O'Callaghan, Pocinki, & Rowe, 2017). A few studies found experimental evidence for central sensitization in the chronification of generalized pain in individuals with JHS or hEDS. The earliest reports concerning central sensitization emerged from studies indicating evidence for generalized hyperalgesia in patients with these conditions. Rombaut et al. (2015) were the first to report the presence of hyperalgesia, also in asymptomatic areas (generalized secondary hyperalgesia), as measured by

TABLE 2 Overview of frequently used modalities of pain testing/investigations in humans (nonexhaustive list)

Clinical sensitivity to innocuous and noxious stimuli				
Sensory test			**Nerve fiber**	**Examples/possible modalities**
Mechanical	Detection threshold	Touch	Aβ fibers	- Monofilaments
				- Cotton swab
				- Brushstrokes
	Pain threshold	Pinprick	Aδ fibers	- Needle
		Pressure	Aδ and C fibers	- Algometer (digital/analog)
Thermal (cold/heat)	Detection threshold		Aδ fibers	- Contact thermode
	Pain threshold		C fibers	
	Paradoxal thermal sensations		Aδ fibers	
Vibration	Detection threshold		Aβ fibers	- Tuning fork
				- Biothesiometer
Electrical	Detection threshold		Aβ fibers	- Skin electrode
	Pain threshold		Aδ fibers	
Pain modulation/processing				
Test			**Pathways**	**Examples/possible modalities**
Nociceptive flexion reflex (NFR)			Spinal nociception Descending pain modulation	- Lower limb NFR - Upper limb NFR
Temporal summation			Pain facilitation	- Mechanical
				- Thermal
				- Electrical
Conditioned pain modulation			Pain inhibition	- Heat/cold as conditioning stimulus and pressure/contact heat as test stimulus
Exercise induced analgesia			Pain inhibition	- Submaximal exercise with pain stimulus (e.g., pressure/ contact heat) before and after
Self-reported pain characteristics				
				Examples/possible modalities
Pain body charts				- Margolis pain diagram
Pain assessment tools			One-dimensional	- Visual analog scale
				- Numeric rating scale
				- Facial pain scale
			Multidimensional	- McGill pain questionnaire
				- Pain disability index
Neuropathic pain assessment				- Pain detect questionnaire
				- DN4 questionnaire
Cognitive-emotional factors				
				Examples/possible modalities
Functional investigations				- Functional magnetic resonance brain imaging
				- Electrophysiological studies (electroencephalography)
				- Positron emission tomography
Questionnaires				- Pain catastrophizing scale
				- Hospital anxiety and depression scale
				- Pain vigilance and awareness questionnaire
				- Central sensitization inventory
				- TAMPA scale for kinesiophobia

pain pressure thresholds (PPTs) in 23 hEDS patients. Di Stefano et al. (2016) also found hyperalgesia to cold and heat in 27 adult JHS/hEDS patients. Scheper et al. (2017) provided evidence for onset of the generalized hyperalgesia in childhood, by studying PPTs in 43 children/adolescents with hEDS/JHS. Experimental research into increased endogenous pain facilitation and/or reduced inhibition, mechanisms underlying central sensitization, is equally scarce in hEDS and JHS/HSD. To date, only three published studies assessed pain facilitation using temporal summation of pain (Benistan & Martinez, 2019; Di Stefano et al., 2016; Leone et al., 2020) and one study examined conditioned pain modulation (Leone et al., 2020) in this patient population. These studies found evidence for increased wind-up to pinprick and thermal stimuli and reduced conditioned pain modulation with thermal stimuli. However, temporal summation of pressure pain and conditioned pain modulation responses for pressure stimuli have not yet been investigated. This is of potential importance in EDS and HSD, since mechanoreceptors are embedded in soft tissue and depend on their deformation for transduction of sensory information. Hypermobility and soft tissue alterations could therefore have an important influence on sensory input and central nervous system processing. Moreover, studies assessing pain inhibition after exercise in EDS and HSD are currently lacking but are of major interest as exercise therapy is a cornerstone in treatment (Engelbert et al., 2017).

Our team recently assessed temporal summation of pressure pain, conditioned pain modulation for pressure stimuli, and exercise induced hypoalgesia in 20 female hEDS patients and 20 healthy controls. We found significantly more wind-up for painful pressure stimuli and significantly less exercise-induced hypoalgesia at the quadriceps (active muscle) in hEDS versus controls. In contrast to Leone et al. (2020), we did not detect differences in conditioned pain modulation between hEDS patients and healthy controls. A possible explanation could be the use of different stimulus types: while Leone et al. (2020) used contact heat as the test stimulus, we used temporal summation of pressure. Still, these data further confirm that central sensitization in hEDS is caused by alterations in endogenous pain facilitation and inhibition (De Wandele et al., 2021).

It has been shown that individuals with hEDS and JHS/HSD frequently experience high levels of anxiety and depression (Baeza-Velasco, Pailhez, Bulbena, & Baghdadli, 2015; Smith et al., 2014) and that these emotions may increase the experience of pain (Linton & Shaw, 2011). Evidence for pain-related fear in hypermobile subjects was provided by Rombaut et al. (2011) who reported fear of falling among women with JHS/hEDS, and Celletti, Castori, La Torre, and Camerota (2013) who reported that kinesiophobia is a common symptom in JHS/hEDS. According to the "fear-avoidance model," a highly fearful person learns to avoid activities that he or she perceives as harmful or pain-provoking (Vlaeyen & Linton, 2000). Over the long term, avoidance behavior that results from fear of pain leads to disuse and muscle deconditioning, which generates further loss of muscle strength and flexibility. This often aggravates functional disability and leads to depression, which in turn reduces pain tolerance and promotes further pain. Besides avoidance strategies, other emotional and cognitive mechanisms likely contribute to chronic pain in EDS and JHS. The current evidence regarding the characteristics and mechanisms of pain in hEDS and JHS/HSD is summarized in Table 3.

4 | THE ROLE OF EXTRACELLULAR MATRIX ALTERATIONS IN PAIN

The study of changes in nociceptive networks that contribute to the development of chronic, maladaptive pain following tissue injury has for a long time, mainly focused on synaptic plasticity in the peripheral (PNS) and central nervous system (CNS), and in changes in the numbers and activity in glial cells. In recent years, increased emphasis has been placed on the environment in which these cells interact, mainly the ECM (Tajerian & Clark, 2015). The ECM consists of collagens, glycoproteins, proteoglycans, elastin, and microfibrillar proteins, which, together with the cells that produce them (e.g., fibroblasts), forms the connective tissue that is found throughout the body (e.g., in skin, vascular wall, bone, tendon, and ligaments; Frantz, Stewart, & Weaver, 2010). An ECM network also surrounds and supports neuronal cells, including nociceptors, and nonneuronal cells in the PNS and CNS (Barros, Franco, & Muller, 2011), where it can regulate synapse formation and function, modulate neuronal excitability, and play a role in CNS neuroplasticity and connectivity (Tajerian & Clark, 2015). Transcriptome analysis in mouse models of nerve injury- and inflammation-induced pain have identified ECM organization as a central molecular pathway in chronic pain development (Parisien et al., 2019). Moreover, peripheral tissue injuries have been shown to cause ECM remodeling and induce pain with alterations in ECM modifying enzymes, such as matrix metalloproteinases (Tajerian & Clark, 2015, 2019).

We and others have provided evidence for alterations in the ECM architecture and organization on dermal biopsies from people with different types of EDS, both *in vivo* (transmission electron microscopy and immunohistochemistry on skin) and *in vitro* (immunofluorescence on dermal fibroblast cultures; Chiarelli, Ritelli, Zoppi, & Colombi, 2019; Hausser & Anton-Lamprecht, 1994; Malfait et al., 2013; Malfait et al., 2010; Syx et al., 2019; Van Damme et al., 2016; Zoppi, Gardella, De Paepe, Barlati, & Colombi, 2004). Therefore, it is possible that an abnormal ECM contributes to the generation and chronification of pain in these conditions, potentially driven by dual factors: the abnormal ECM may (a) compromise the proper development and/or support of the (peripheral) nervous system, contributing to increased vulnerability of the peripheral nerves; and (b) induce molecular and/or cellular changes in the nociceptors and the PNS. It has been reported that a variety of sequestered ECM components or their degradation products can act as damage-associated molecular patterns (Schaefer, 2014), which function as endogenous danger signals and are recognized by pattern recognition receptors (PRRs) of the immune system. Sensory neurons have been shown to express the same PRRs (e.g., Toll-like receptors), which can induce painful pathways upon activation (Feldman, Due, Ripsch, Khanna, & White, 2012; Lacagnina, Watkins, & Grace, 2018; Miller et al., 2015; Qi et al., 2011). It could be hypothesized that

TABLE 3 Overview of studies assessing pain in EDS patients

Study	Cohort	Tests	Findings
Sachetti et al. (1997)	51 EDS patients (13 cEDS, 29 hEDS/JHS, 7 vEDS, 2 unknown)	Questionnaires (MPQ) and interview	Pain (>6 months) in 90% Interference with sleep and physical activity (70%)
Rombaut et al. (2010)	32 JHS/hEDS patients and 32 sex- and age-matched controls	Vibration detection threshold	No significant differences
		Joint position sense	Significant impairment in knee joint repositioning
Voermans et al. (2011)	273 EDS patients (53 undefined EDS, 45 cEDS, 162 hEDS/JHS, 11 vEDS, 2 kEDS)	Questionnaires (MPQ, SIP, SC subscale sleep, CIS subscale fatigue, SF-36, SIP)	Pain in 90% (98% JHS/hEDS versus 76% cEDS and 55% vEDS) Higher pain scores in JHS/hEDS, 50% of the patients with pain suffered sleep disturbances and 87% was impaired in performing daily activities
Camerota et al. (2011)	44 EDS patients (29 JHS/hEDS and 15 cEDS)	Questionnaires (NRS, the ID Pain, NPSI)	89% complained of at least moderate pain At least "probable" neuropathic pain in 68%
Rombaut et al. (2015)	23 JHS/hEDS patients and 23 sex- and age-matched controls	PPT	Significantly lower PPTs
		Questionnaires (Margolis pain diagram, PDQ, HADS, PCS, PVAQ, CIS, SF-36)	Predominantly neuropathic pain component in 50% and significantly higher percentage of painful body surface. Significant correlations between PPTs and psychosocial factors, fatigue, disability, health status and duration of the symptoms
Cazzato et al. (2016)	20 JHS/hEDS, 3 vEDS, 1 cEDS	IENFD	All showed a decreased IENFD consistent with the diagnosis of small fiber neuropathy
		Sural nerve conduction studies	Normal in all individuals
		Questionnaires (NRS, DN4, ID pain, SFN-SIQ)	Moderate pain intensity (NRS >4 and <7) in 33% and severe (NRS >7) in 46% Neuropathic pain symptoms in 96%
Scheper et al. (2016)	74 JHS/hEDS patients, 74 GJH without chronic pain, and 77 healthy controls	PPT	Significantly lower PPTs
		Questionnaires (VAS, multidimensional fatigue scale of the pediatric quality of life inventory, revised Child anxiety and depression scale, CIS, HADS)	Generalized hyperalgesia was found to be an independent variable not related to the amount of painful body surface, fatigue, or psychological distress
Di Stefano et al. (2016)	27 hEDS/JHS	Mechanical detection threshold with monofilaments	Sensory profiling disclosed No sensory deficits
		Vibration detection threshold	
		Pinprick sensation	
		Thermal detection and pain thresholds	Lowered cold and heat pain thresholds
		Nerve conduction study	No significant differences (compared to reference values)
		Laser-evoked brain potentials	No significant differences (compared to reference values)
		Temporal summation to repetitive pinprick stimuli	Increased wind-up ratio
		Questionnaires (widespread pain index, DN4, and the fibromyalgia rapid screening tool)	Most subjects suffered from widespread pain The DN4 questionnaire argued against neuropathic pain The fibromyalgia rapid screening tool score was compatible with fibromyalgia in 88%

(Continues)

TABLE 3 (Continued)

Study	Cohort	Tests	Findings
Benistan et al. (2019)	37 hEDS patients	Brush-induced allodynia	Cutaneous hyperesthesia in 54%
		Thermal detection and pain thresholds	Hypoesthesia to thermal stimulation in 26% but no thermal hyperalgesia
		Vibration detection threshold	Asymmetry in the vibratory perception threshold in 32%
		Wind-up with monofilaments	Increased wind-up ratio in 37%
		Questionnaires (BPI short form, MPQ, DN4, NPSI, HADS)	Severe/moderate pain in 67%, important impact of pain on quality of life. Arguments for neuropathic pain in 67%, suspected depression in (15%) and anxiety in 7.5%
Leone et al. (2020)	22 hEDS and 22 sex- and age-matched controls	Mechanical detection threshold with monofilaments	Sensory profiling disclosed No sensory deficits
		Vibration detection threshold	
		Pinprick sensation	
		Thermal detection and pain thresholds for cold and heat	No significant differences
		Mechanical pain threshold and wind-up with pinprick stimuli	Increased wind-up ratio
		CPM (contact heat as test and conditioning stimulus)	The test stimulus rating increased during conditioning in patients
		Questionnaires (widespread pain index, DN4)	Most subjects suffered from widespread pain. In all patients, the DN4 questionnaire argued against neuropathic pain
De Wandele et al. (2021)	20 hEDS patients and 20 matched controls	Thermal detection and pain thresholds	No significant differences
		PPT	PPTs were significantly lower
		Wind-up with pressure stimuli	Increased wind-up for mechanical stimuli
		Exercise-induced analgesia with pressure stimuli	Submaximal exercise-induced significantly less hypoalgesia at the quadriceps
		CPM (conditioning stimulus: hot water immersion, test stimulus: pressure stimuli)	No significant differences
		Questionnaires (Margolis pain diagram, PDQ, HADS, PCS, PVAQ, TAMPA scale kinesiophobia)	The questionnaire results suggested cognitive emotional sensitization

Abbreviations: cEDS, classic EDS; CIS, Checklist Individual Strength; CPM, conditioned pain modulation; GJH, generalized joint hypermobility; HADS, Hospital Anxiety and Depression Scale; hEDS, hypermobile EDS; IENFD, intraepidermal nerve fiber density; JHS, joint hypermobility syndrome; kEDS, kyphoscoliotic EDS; MPQ, McGill Pain Questionnaire; NPSI, Neuropathic Pain Symptom Inventory; PCS, Pain Catastrophizing Scale; PDQ, Pain Detect Questionnaire; PPT, Pain pressure threshold; PVAQ, Pain Vigilance and Awareness Scale; SC, Symptom Checklist; SFN-SIQ, Small Fiber Neuropathy and Symptoms Inventory Questionnaire; SIP, Sickness Impact Profile; VAS, Visual Analog Scale; vEDS, vascular EDS.

continuous stimulation of peripheral nociceptive termini that innervate damaged tissues by mediators released from a disorganized ECM seen in EDS leads to altered gene transcription and protein expression in the nervous system. These molecular and/or cellular changes may result in repeated or chronic activation of nociceptors and induce neuroplasticity changes, contributing to sensitization and the generation and maintenance of chronic pain. This hypothesis however remains to be proven by experimental data.

5 | THE STUDY OF PAIN MECHANISMS IN MOUSE MODELS OF EDS

Because experimental pain studies in people with EDS are limited by several factors, such as ethical considerations, their subjective nature, and difficulties in obtaining neuronal tissues and cells for molecular studies, animals, most commonly mice (*Mus musculus*) and rats (*Rattus norvegicus*), are widely used as models to study pain (Mogil, 2009).

TABLE 4 Overview of commonly used stimulus-evoked and nonstimulus-evoked methods to study pain behavior in rodent animal models (nonexhaustive list)

Stimulus-evoked methods		
Mechanical		Manual or electronic von Frey fibers
		Randall-Selitto algesiometer
		Calibrated forceps
Thermal	Heat	Tail–flick test
		Hot plate test
		Hargreaves test
		Thermal probe test
	Cold	Cold plate test
		Cold plantar test
		Acetone evaporation test
	Preference	Temperature preference assays as a surrogate measure for thermal aversion
Chemical		Formalin, acetic acid, complete Freund's adjuvant injection in hind paw or abdominal area
Nonstimulus-evoked methods		
Grimace scales		
Weight-bearing analysis		
Gait analysis		
Automated analysis of spontaneous behavior (e.g., distance traveled, climbing, drinking/eating)		
Burrowing and nesting assays		

Animal models of nociception date back to the late 19th century and have been crucial in our understanding of pain processes and in the discovery of pain-relieving therapies (Gregory et al., 2013; Minett, Quick, & Wood, 2011).

Since animals cannot report pain and thus pain cannot be directly evaluated in rodents, a range of indirect behavioral methods have been developed to reliably assess and quantify signs of nociception in these noncommunicating subjects. These methods are divided into stimulus-evoked (using either mechanical, thermal, or chemical stimuli) methods, and stimulus-independent methods (Table 4, reviewed in Deuis, Dvorakova, & Vetter, 2017; Gregory et al., 2013; Turner, Pang, & Lofgren, 2019).

Research into pain-related behaviors in animal models of EDS is only in its infancy. Several mouse models of molecularly elucidated EDS types have been generated and characterized over the past few decades (Vroman et al., 2021). While these models have been found to provide valid models for their human counterparts, only two studies have assessed pain behavior in EDS mouse models (Table 5). Recently, our group reported that both male and female *Col5a1*$^{+/-}$ mice, a model for classical EDS (Wenstrup et al., 2006), are hypersensitive to mechanical stimuli in the hind paws and abdominal area but not to thermal stimuli. In addition, female *Col5a1*$^{+/-}$ mice showed altered climbing behavior with decreased grip strength. Visualization

TABLE 5 Overview of pain-related behaviors and findings assessed in mouse models of EDS

	Col5a1$^{+/-}$	*Tnxb*$^{-/-}$
EDS type	Classical EDS	Classical-like EDS
Mechanical sensitivity	Increased (mechanical allodynia) [von Frey: hind paw, abdominal area]	Increased (mechanical allodynia) [von Frey: hind paw; cotton swab: hind paw (not significant)]
Thermal sensitivity	Normal [cold plate (0°C); hot plate (55°C)]	Normal [acetone evaporation test (cold): hind paw; hot plate (55°C)]
Chemical sensitivity	NE	Increased [formalin injection: hind paw, male mice]
Electrical sensitivity	NE	Increased (myelinated Aδ and aAβ fibers) [transcutaneous sine wave stimuli, male mice]
Spontaneous behavior	Normal distance traveled Normal rearing Decreased climbing time in female mice [LABORAS platform]	NE
Other behavioral alterations	Decreased grip strength (only significant in female mice when corrected for body weight) (surrogate measure for deep tissue hyperalgesia) [grip strength meter: forepaws] Increased audible vocalization upon scruffing	NE
Cutaneous innervation	Disorganization of the $Na_V1.8$-positive fibers with less protrusion of the nerve fibers into the epidermis [*Col5a1*$^{+/-}$ x Na_V1.8-tdTomato reporter mice: glabrous skin of the hind paw]	NE
Dorsal horn	NE	Increased number of P-ERK and NAPDH-diaphorase positive neurons (male mice)

Note: Experiments were performed in both male and female mice unless otherwise stated. The techniques used are depicted between brackets.
Abbreviations: EDS, Ehlers–Danlos syndromes; NE, not examined.

of nociceptors in the glabrous skin of the footpad revealed a decreased intraepidermal nerve fiber density, with fewer nerves crossing the epidermis and a reduced total nerve length of *Col5a1*$^{+/-}$ mice compared to wild-type (Syx et al., 2020).

At the same time, Okuda-Ashitaka et al. (2020) studied *Tenascin X* knockout (*Tnxb*$^{-/-}$) mice, a model of classical-like EDS, and also found mechanical allodynia but no thermal hypersensitivity. A chemical pain stimulus with formalin injection in the hind paw evoked an increased pain response during the first (acute) phase (0–5 min) and early during the second (inflammatory) phase (16–30 min) in *Tnxb*$^{-/-}$ mice, compared to wild-type. Transcutaneous sine wave stimuli elicited a hypersensitive response of myelinated Aδ and Aβ fibers, but not of unmyelinated C fibers. In addition, *Tnxb*$^{-/-}$ mice showed an increased number of P-ERK and NADPH-diaphorase positive neurons in the dorsal horn of the spinal cord, suggestive of spinal central sensitization (Okuda-Ashitaka et al., 2020).

Together, these findings illustrate that the nervous system and associated pain-related behaviors are compromised in mouse models of EDS, and further highlight the potentially important role of an intact ECM for proper innervation and pain processing. In addition, these models will provide useful tools to pave the way to a better understanding of the mechanisms of pain associated with EDS, which may ultimately lead to the discovery of new targets for the development of effective therapeutic interventions.

6 | KNOWLEDGE GAPS AND OPPORTUNITIES FOR FUTURE RESEARCH

Pain is undoubtedly a major problem in individuals suffering from hEDS and JHS/HSD and is associated with increased morbidity, reduced functionality, and reduced quality of life, and with increased use and abuse of pharmacological and nonpharmacological pain-relieving treatments. Yet these treatments often only have a moderate effect at best and may be associated with severe complications and health risks.

To date, virtually no experimental pain studies have been performed in people with molecularly elucidated forms of EDS. This could be because for those conditions, research has mainly focused on other complications, such as vascular fragility, or it could also be that they experience less pain than people with hEDS or JHS. Yet, the multisystemic connective tissue fragility in these people also predestines them to repeated micro- and macrotrauma. In addition, since most studies on hypermobility-related pain focused on hEDS/JHS patients, and since the diagnosis of these conditions is much more frequent in females than in males, most information on pain prevalence and pain mechanisms has been gathered in adult females and little information is available on pain prevalence and mechanisms in male and/or pediatric/adolescent populations with molecularly elucidated types of EDS or with HSD. Further studies are needed to elucidate pain prevalence and pain mechanisms (nociceptive, neuropathic, nociplastic) in these populations to provide insights in the sex-specific natural history of chronic pain in these conditions, and its association with the underlying molecular defects.

The exact reason for the sex-divergence observed for hEDS and JHS/HSD remains currently elusive. Intriguingly, sex differences in chronic pain prevalence have been well established (Mogil, 2020). It has been widely reported that the prevalence rates of many different pain conditions are higher in women than in men, and that chronic pain is usually more severe for women than men with the same disease condition (Kim & Kim, 2020). The higher prevalence of hEDS/JHS in females could therefore, at least partly, be attributed to the presence of chronic pain, since this a major criterion for the diagnosis of hEDS and JHS, but this remains to be proven. Research regarding the mechanisms governing sex-related differences in pain in general is still in its infancy due to the bias in biomedical research, which has largely been performed in males. Studies comparing pain behavior and mechanisms between males and females with different forms of EDS and HSD are therefore also needed to provide insights into sex differences in pain prevalence and mechanisms in these conditions.

Animal models, especially rodents, have been great allies in advancing our insights into the pathophysiological mechanisms of disease and can serve as preclinical models for the development and assessment of pharmacological treatments. Also in the field of pain research, many advances have occurred in rodent pain assessment techniques. Besides behavioral tests (see Table 4), several other state-of-the-art techniques that can help increase our understanding of mechanisms underlying pain processing and modulation. These include for example neuroimaging techniques (e.g., functional magnetic resonance imaging of murine brains; Da Silva & Seminowicz, 2019), and transgenic animals which facilitate visualization of specific neuronal subsets (e.g., $Na_V1.8$ positive neurons; Gautron et al., 2011; Obeidat, Miller, Miller, & Malfait, 2019; Shields et al., 2012) or their activities (e.g., genetically encoded fluorescent calcium indicator GCaMP to perform *in vivo* calcium imaging; Emery et al., 2016; Miller et al., 2018). In addition, several neurobiological techniques have been developed that allow the modulation of particular groups of neurons to elucidate their role in different aspects of the pain response. Optogenetic approaches use light-sensitive (photoactivatable) stimulatory or inhibitory ion channels (opsins) whereas chemogenetic approaches, for example, "Designer Receptors Exclusively Activated By Designer Drugs" (DREADDs), use engineered G-protein-coupled receptors (GPCRs) to activate or inhibit neurons upon binding of synthetic chemicals (Jayaraj et al., 2018; Miller et al., 2017; Urban & Roth, 2015). Furthermore, advances in molecular techniques, such as proteomics and transcriptomic approaches, are also increasingly applied in the neuroscience and pain research field, and have led for example to the classification of several neuronal types by single-cell RNA sequencing (Usoskin et al., 2015), and to defining temporospatial pain-related signatures.

For most EDS types, one or several mouse models have been developed (Vroman et al., 2021). Recent observations of altered pain-related behaviors and innervation in the classical and classical-like EDS mouse models underscore their potential as preclinical models to investigate and unravel the pathways and mechanisms underlying EDS-related pain, opening up an important area for future research. Research into pain behavior and mechanisms in these models, both in

males and females, is urgently needed, as it will increase our understanding of the complex interplay between ECM alterations, nervous system development, and function as well as initiation and maintenance of chronic pain. It may also shed light on mechanisms underlying chronic pain in hEDS and HSD, for which currently no animal models are available, and even other common chronic pain conditions such as fibromyalgia.

Although rodent (mammalian) animal models are dominating research on nociception and pain, increasing evidence points to comparable processes in fish. The tropical freshwater teleost or bony fish, *Danio rerio* (zebrafish), is widely used as an (vertebral) animal model in many research areas such as disease modeling, regeneration, and toxicology. Its popularity is fueled by several advantages, such as small size, easy genetic manipulation, high fecundity, external fertilization, large offspring, transparency of rapidly developing embryos, and low maintenance cost. Initial discussions questioned whether (zebra)fish were able to perceive pain, but the identification of nociceptors using electrophysiology and neuroanatomical approaches in rainbow trout (also a teleost) together with other mounting evidence changed this view (Sneddon, 2002, 2003, 2015). Also for zebrafish, it is now clearly established that they can perceive pain, both in early and adult stages, and this model is increasingly used in neurobiological and nociceptive research (Malafoglia, Bryant, Raffaeli, Giordano, & Bellipanni, 2013). Methods to assess behavioral changes in response to stimuli of chemical, thermal, and electrical origin have been developed to model both acute and ongoing (chronic) pain in zebrafish (Ohnesorge, Heinl, & Lewejohann, 2021). In addition, electrophysiology, optogenetic tools, and transgenic lines that label specific neuronal subsets are also available in the zebrafish field. Furthermore, zebrafish can facilitate the screening for potent analgesics in translational pain research by allowing larger throughput compared to other vertebrate models (Kalueff et al., 2016). Recently, the use of zebrafish in EDS research has been introduced by our lab with the description of several zebrafish models for cardiac-valvular EDS (*col1a2*$^{-/-}$; Gistelinck et al., 2018) and spondylodysplastic EDS (*b4galt7*$^{-/-}$, Delbaere, Van Damme et al., 2020; and *b3galt6*$^{-/-}$, Delbaere, De Clercq et al., 2020), which mimic features of the respective human EDS counterparts. As such, these models provide an excellent opportunity to start addressing EDS-related pain in zebrafish and might expedite our research on unraveling the basic mechanisms and neuronal alterations that contribute to pain in these EDS types as well as evaluate disease-tailored therapeutic options.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

DATA AVILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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